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NEW FACETS IN COORDINATION CHEMISTRY: THE SPONTANEOUS AUTOXIDATION OF DOPAMINE AND THE INVOLVEMENT OF METAL IONS IN THE PROGRESS OF DEGENERATIVE MENTAL DISEASE

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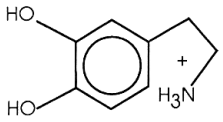
Abstract

A detailed kinetic study has been carried out of the reaction of dopamine, 2-(3,4-dihydroxyphenyl)-ethylamine, with dioxygen over the pH range 7-9, where it reacts spontaneously without the necessity of metal-ion catalysis. Stoichiometric amounts of H_2O_2 were shown to be produced. The other product of oxidation is, initially, the pink dopaminochrome which is not stable and reacts further (without the consumption of dioxygen) to form the insoluble polymeric material known as "melanine". The rate determining step is assumed to be hydrogen atom abstraction from the monodeprotonated species by O_2. Based on these results the *in vitro* chemistry of the reactions of dopamine (DA), 5-hydroxydopamine (5-OHDA), and 6-hydroxydopamine (6-OHDA) under the presence of iron(III) and dioxygen has been studied. The reaction pathway then essentially involves a FeL intermediate, which decomposes releasing Fe(II) and the above mentioned dopaminochrome, which reacts further under involvement of both Fe(III) and dioxygen. The important relevance of these reactions to the development of Parkinson's disease is examined. A mechanism for its initialisation and its progress is suggested by which the presence of excess iron(III) could arise and its consequences are discussed.

Key Words: Parkinson's disease, dopamine, 6-hydroxydopamine, iron(III), iron(II), manganese(II).

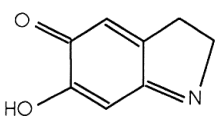
Introduction.

Chemical Aspects: Dopamine is 2-(3,4-dihydroxyphenyl)-ethylamine, **I**, and is referred to as H_2LN^+ in this paper where the phenolic protons are written to the left of L. When a neutral solution of dopamine is



I

exposed to air, after a while it turns pink due to oxidation to dopaminochrome, **II**, even in the absence of metal ions. Finally, the pink colour disappears to be replaced by a precipitate of the polymeric material, melanine. Addition of a small amount of acid inhibits this oxidation, unless metal ions such as Fe^{3+}, Cu^{2+} or VO^{2+} are present



II

However, the markedly different behaviour of the closely related – both chemically and biochemically – catecholamines adrenaline (1-(3,4-dihydroxyphenyl)-2-(methylamino)ethanol)) and dopa (3-(3,4-dihydroxyphenyl)-alanine) should be noted. In the rigorous absence of metal ions they are stable even in neutral solution. In acid solution (pH<4-5) the catalyzed oxidation of dopa proceeds smoothly to completion[1]. In contrast, in the case of dopamine, although added metal ions initially start an oxidation process, this soon comes to an end as the metal ions are efficiently removed from the solution by the melanine (or a soluble, polymeric, precursor). The involvement of metal ions in this reaction is being studied in greater detail[2].

Medical Aspects: Some populations of melanized dopaminergic neurons of the brain stem have been shown to be selectively vulnerable[3]. They show an increased rate of degeneration in both normal ageing and in the development of Parkinson's Disease (PD) in contrast to most other neurons of the lower brain stem[4].

These dopaminergic neurons are also specifically sensitive to manganese and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) as well as to iron and 6-hydroxydopamine (6-OHDA) and to the (as yet unidentified) factors causing PD[5]. In this respect the apparent interplay between «pro-neurotoxins» such as MPTP and 6-OHDA that can be converted by monoamine oxidase B (MAO B) into neurotoxins is not yet fully understood. It has been suggested that an alternative mechanism for the alleged anti-degenerative action of the MAO B inhibitor, deprenyl, could be the inhibition of the formation of H_2O_2 and oxygen radicals by MAO[6]. The destruction of pigmented brain stem nuclei, particularly those of the *Substantia Nigra* (SN) appears to be the key to the pathogenesis and pharmacology of PD[7]. Although the cause of dopaminergic cell death remains unknown, excess iron within the SN has been implicated in the progression of the disease through its participation in the continuous formation of cytotoxic free radicals[8].

Based on the demonstration of increased Fe(III) in the SN of the brains of deceased patients suffering from PD together with its accumulation on melanin particles in the remaining cells[9], it has recently been proposed[10] that iron-melanin interactions may be involved in the degeneration of dopaminergic neurons. A possible mechanism that leads to an increased level of iron, not protected by being bound to ferritin, is thus required. This decompartmentation (or tissue iron overload) is already known to lead to a state of oxidative stress[11], and it is highly relevant that many cytotoxic compounds that readily promulgate oxidation-reduction reactions can release iron from storage. A well documented example is

that of paraquat toxicity of the lung which has been linked to the ability of paraquat to completely release iron from ferritin[12]. Studies have demonstrated that, in rats, intraventricular pre-injection of desferral (a selective iron chelator) attenuates 6-OHDA-induced lesions of nigrostriatal dopamine neurons[11] This was made evident by the prevention of loss of striatal dopamine (DA) and the reduction of homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC) (usually seen after injection of 6-OHDA). Seen in this light, the following *in vitro* studies would seem to shed some light on the role of iron and 6-OHDA in the progression of PD:

Firstly it is shown that uncomplexed Fe(II) is able to interact with H_2O_2 promoting a Fenton-like reaction and forming the (cytotoxic) hydroxyl radical which then reacts with dopamine to yield 5-OHDA and 6-OHDA. Secondly, comparison of the aqueous coordination chemistry of iron(III) with DA, 5-OHDA, or 6-OHDA as potential ligand was studied by stopped-flow spectrometry by which it is demostrated the 6-OHDA alone is capable of releasing iron from strongly complexing species.

Finally, kinetic studies of the reaction between dopamine and dioxygen with and without the presence of iron(III) revealed that neither melanin nor iron-containing melanin played a part in this reaction and that in fact iron(III) is not a catalyst for this reaction (although it can *initiate* the reaction, even in acid media).

Therefore dopamine has attracted much interest due to its postulated role in Parkinson's and Alzheimer's diseases[13,14]. Especially, the mentioned role of the products of its oxidation and the role of iron-containing melanines found in the *substantia nigra* of the brains of deceased Parkinsonian patients[5,10,15,16]. This, together with our interest in the role of coordination compounds as catalysts for autoxidation reactions, has stimulated the present study.

Experimental

Dopamine (3-Hydroxytyramine or DA) Hydrochloride ($C_8H_{11}NO_2$.HCl) was supplied by Sigma Chemical Co. (St. Louis, MO) and used without further purification. The ionic strength was adjusted with potassium chloride (Merck, *pro analysi)* to be 0.100 mol dm^{-3} in chloride ion; the use of nitrate ion as background electrolyte gave identical results. 5-OHDA, and 6-OHDA as hydrochlorides, were obtained from Sigma (St. Louis, MO, U.S.A). Whenever possible analytical grade reagents were used. Synthetic melanins were prepared by autoxidation according to Das et al. [17].

Iron(III) nitrate nonahydrate, manganese(II) sulphate monohydrate, coppert(II) nitrate, Oxovanadium(IV) sulphate, sodium *di*-hydrogen phosphate, *di*-potassium hydrogen phosphate were from Merck and EPPS-buffer (3-(4-(2-hydroxyethyl)-1-piperazino)-propanesulfonic acid was obtained from Aldrich: all were of *pro analysi* quality. Catalase (from bovine liver) and superoxide dismutase (SOD; from bovine erythrocytes) came from Sigma.

Oxygen consumption was measured using a Clark-type electrode (EO 96, WTW) connected to a corresponding processor unit (Oxi 537, WTW). The output of the processor unit was connected to a Goerz-Metrawatt x-t-recorder. The pH was kept constant (within ±0.003 units) with an RTS 822 titrating unit (Radiometer, Copenhagen) coupled with a PHM 84 pH-meter and the amount of added base was recorded. The temperature was held constant within ±0.1 °C by a K2 Ultrathermostat (Lauda). In a thermostatted glass vessel equipped with a lid that held the pH and oxygen electrodes and inlet tubes (the lid was closely fitting but could be slid up and down) 110 ml of the slightly acidified dopamine solution were saturated with oxygen until a constant value of the oxygen concentration was reached (usually within 10-15 minutes). Then the oxygen tube was removed, the lid lowered until it touched the surface of the solution and the reaction started by automatic addition of base (the desired pH was established in less

than two minutes). The solution was kept stirred (necessary for the correct functioning of the oxygen electrode) by use of a circular teflon non-vortexing magnetic stirrer supplied by Watman Lab-Sales. Occassionally a sample was withdrawn in order to record a UV-Vis spectrum (Hitachi U-2000 spectrometer).

Additional spectra, together with the kinetics of formation of dopaminochrome, were obtained with a Tracor Northern diode array high-speed-spectrophotometer, supplied by Applied Photophysics Ltd. (London). These stopped-flow data were recorded by mixing slightly acidified dopamine solutions with alkaline phosphate buffer, both saturated with oxygen.

Attempts to estimate hydrogen peroxide by Marklund's modification[18] of Bonet-Maury's Ti(lV) reagent [19] failed due to the general absorption of melanin.

The reversed-phase high performance liquid chromatographic (HPLC) determinations were carried out using a Model 5000 solvent delivery module (ESA, Bedford, MA, USA) and a Coulochem 5100 A detector (ESA) with a 5010 analytical cell (ESA) connected to a Cl-10 recording integrator (LDC/Milton Roy Riviera Beach, FL, U.S.A.). Potential differences were +0,46 V and –0,22V respectively. Samples were introduced using a 100ml injection loop. Separations were carried out using a 250x4,6mm ID SUPELCOSIL LC-18 (5mm average particle size) column. The mobile phase (pH=3) contained 20 mM potassium dihydrogen phosphate, 0,4mM octanesulphonic acid and 14% methanol (v/v). The flow-rate of the mobile phase was 1,0 ml/min. Prior to use, the mobile phase was filtered through a 0,22 mm membrane filter (Sartorius, Göttingen, FRG). Peak identifications were performed by admixture with authentic standards.

Results

Protonation Constants of Dopamine. The protonation equilibria for dopamine are listed in (1) together with the

values of the protonation constants reported by Gergely and Kiss[20] (note that dopamine is written here as H_3L^+ as these are macroconstants and cannot therefore be assigned).

$$L^{2-} + H^+ \Leftrightarrow HL^- \log K_1^H = 13.1$$

(1) $$HL^- + H^+ \Leftrightarrow H_2L \log K_2^H = 10.41$$

$$H_2L + H^+ \Leftrightarrow H_3L^+ \log K_3^H = 8.82$$

The microconstant, K_m^{OH}, for the protonation of HLH (i.e. the addition of the second phenolic proton whilst the amino group is protonated) was also determined by these authors ($\log K_m^{OH}=8.87$).

Kinetics. The consumption of oxygen is accurately first-order in oxygen for over three half-lives and is independent of ambient light. Phosphate buffer leaves the rate unchanged and this enabled the investigation of the reaction in a stopped-flow spectrometer. Borax buffer could not be used because it inhibits the reaction by forming a compound with the catechol function of dopamine, while EPPS-buffer was also unsuitable because it strongly accelerates the reaction. Typical first-order rate constants, k^{obs}, are collected in Table1 and data for 25°C shown in Fig. 1. The addition of base was also first-order and the rate constants so obtained were identical to those calculated from the oxygen measurements (Table 1).

As demonstrated in the Fig. 2, the rate constants are inversely proportional to $[H^+]$ and directly proportional to the total concentration of dopamine, $[L]_T$. The rate law is thus given by (2), and k^{obs} can be expressed by (3) in which A is a constant.

(2) $$-d[O_2]/dt=k^{obs}[O_2]$$

(3) $$k^{obs}=A[L]_T/[H^+]$$

Some calculated values of A are given in Table 1 with a mean value of $(1.27\pm0.09)\times10^{-9}$ s^{-1} at 25°C and at an ionic

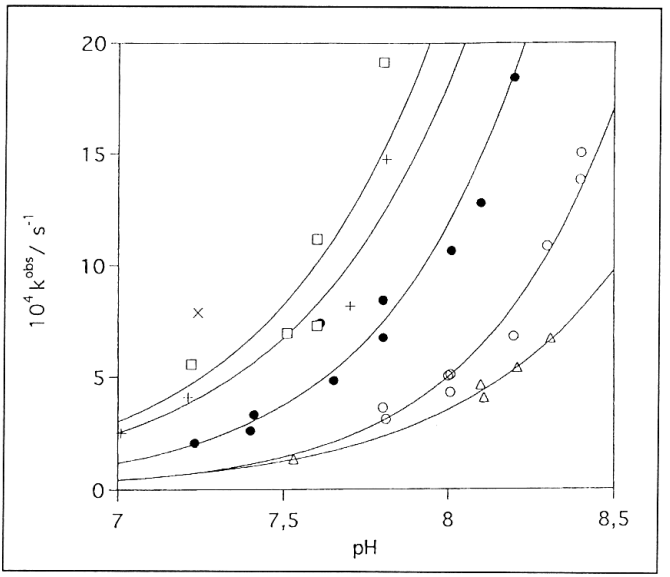


Fig. 1 - Typical first order rate constants for the autoxidation of dopamine. Experimental conditions: 298 K, I=0.100 mol dm^{-3}, $[L]_T$=Δ 0.003, o 0.005, • 0.01, + 0.02, 0.03 and x 0.04 $mol.dm^{-3}$.

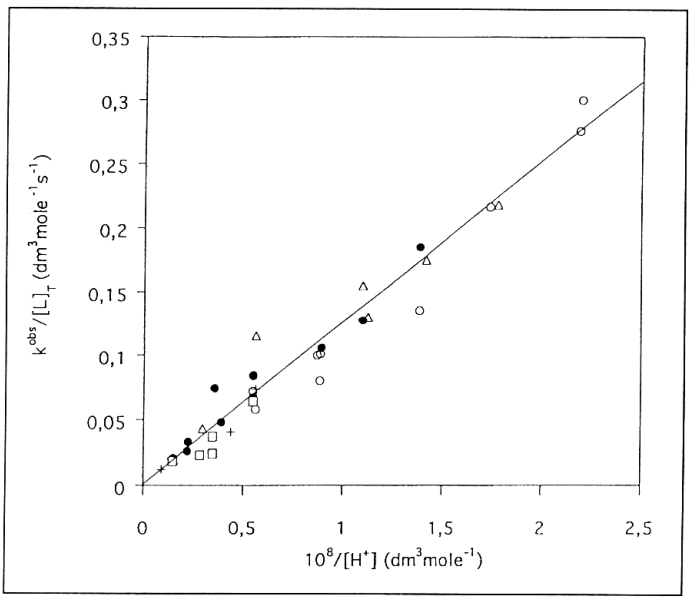


Fig. 2 - $k^{obs}/[L]_T$ versus $1/[H^+]$. The slope of the regression line gives $A=1.27.10^{-9}$ s^{-1}. Symbols are the same as in Fig. 1.

Table 1 - Typical observed rate constants for the consumption of oxygen ($k^{obs}(O_2)$) and for the addition of base ($k^{obs}(OH^-)$), the amount of based added ($n(OH^-)$), and A-values calculated according to Eq. (3).

T °C	pH	$[L]_T$ mmole dm^{-3}	$k^{obs}(O_2)$ $10^{-3}s^{-1}$	$k^{obs}(OH^-)$ $10^{-3}s^{-1}$	$n(OH^-)$ µmoles	A $10^{-9}s^{-1}$
7.2	8.60	5.05	0.323			0.183
	8.21	10.0	0.220			0.155
	8.40	10.0	0.383			0.175
	8.65	10.0	0.791			0.203
	8.81	10.0	1.37			0.246
	8.60	20.0	1.37			0.198
						0.19±0.03
15.0	8.22	5.05	0.210			0.286
	8.00	10.0	0.248			0.282
	8.18	10.0	0.354			0.268
	8.40	10.0	0.672			0.307
	8.61	10.0	1.23			0.345
	8.20	20.0	0.698			0.251
						0.29+0.03
25.0	8.31	3.07	0.668	0.664	55.8	1.24
	8.00	5.00	0.499	0.434	67.6	1.15
	7.65	9.98	0.495	0.418	57.8	1.28
	8.01	10.0	1.06	1.04	53.6	1.20
	8.10	9.97	1.27	1.08	71.5	1.16
	8.20	9.97	1.84	1.62	66.1	1.33
	7.80	20.1	1.47	1.24	67.6	1.33
	7.23	30.0	0.556	0.445	63.7	1.28
	7.80	30.0	1.80	1.91	70.2	1.17
	7.25	40.0	0.791	0.848	59.7	1.29
	7.41	9.84	0.338			1.45
	7.80	10.0	0.484	0.495	67.0	0.87[b]
	8.00	10.1	0.802			0.90[c]
	7.20	20.0	1.23			1.69[d]
	7.20	20.0	1.22			1.68[e]
	7.67	12.9	0.956			1.82[f]
	8.40	5.00	1.37^			1.26[g]
						1.27±0.09

strengh of 0.100 mole dm^{-3} (Cl^-). The variation of A with temperature establishes an apparent activation energy of 75 kJ $mole^{-1}$.

The addition of methanol, which is known to act as a radical trap, results in a 30% decrease in k^{obs}. The addition of SOD has no effect on the rate, suggesting that the superoxide ion is not a chain carrier in this reaction. Catalase, when added after the reaction has proceeded for two half-lives, recovered about 50% of the oxygen, consumed up to this point, thus confirming peroxide to be the final product of oxygen reduction.

Fig. 3 illustrates the route by which dopamine is converted to dopaminochrome.

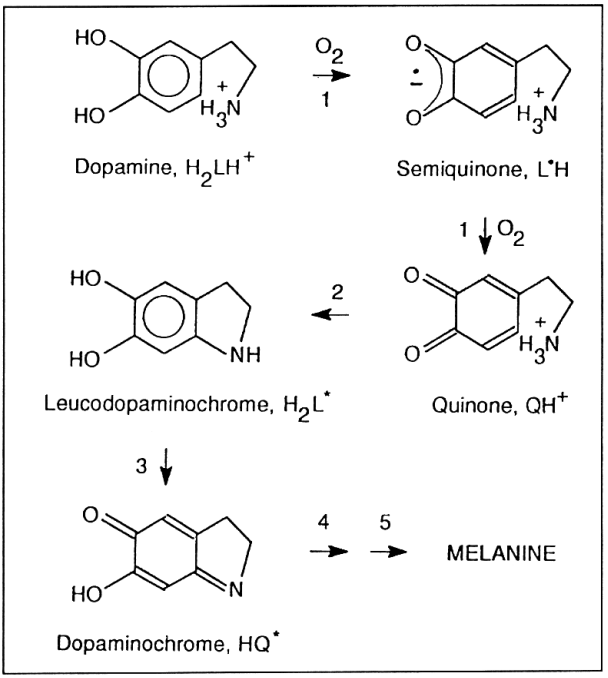


Fig. 3 - The course of the autoxidation of dopamine. 1... One-electron-oxidation; 2... Cyclisation *via* Michael addition; 3... Two-electron-oxidation; 4... Formation of soluble melanine-precursors; 5... Polymerisation.

The amount of added base (calculated from the intercept of the first-order plot) is independent of pH and dopamine concentration and for initially saturated oxygen solutions was found to be 0.061±0.008 mmoles. This is approximately half the initial amount of oxygen (0.132 mmoles[21]), *ie.* one proton is released for every two oxygen molecules consumed. Thus the overall reaction can be written as (4), dopaminochrome acting as an acid in this pH-range.

(4) $$H_2LH^+ + 2O_2 \rightarrow Q^{\bullet -} + H^+ + 2H_2O_2$$

The expression for k^{obs} above, (3), strongly suggests that the reaction being followed is the rate-determining abstraction of a hydrogen atom from the monodeprotonated dopamine, HLH, (5) and that all subsequent steps depicted

(5) $$HLH + O_2 \xrightarrow{k_1} L^{\bullet}H + HO_2^{\bullet}$$

in Fig. 3 are fast. In other words, after deprotonation of one ot the hydroxy groups of dopamine, the molecule reacts in the rate determining step with oxygen to give the semiquinone radical and superoxide ion. The subsequent reaction of the semiquinone with another molecule of oxygen yielding the *ortho*-quinone is fast[22] and therefore is the most likely step to involve the second oxygen molecule. Although the disproportionation of superoxide anions is extremely slow, the reaction of the anion with the conjugated acid is very fast – fast enough to compensate for the very low concentration of the protonated form[22] ($\log K_a \approx 4.8$). This finding is in accordance with the observations[23] of Misra and Fridovich for the autoxidation of adrenaline, where it was found that two different chains are acting: one based on the semiquinones acting as chain carriers and the other based on superoxide radicals, the latter predominant at higher pH. They discriminated between the two pathways by the amount of inhibition by

superoxide dismutase. However, they demonstrated that below pH 9 the reaction proceeds exclusively *via* the semiquinone path. Also the cyclisation of dopaminoquinone is known to be fast compared to the initial electron transfer in the pH-range employed[24]. Since dopaminochrome is considered a final product of the oxygen-consuming and the proton-releasing reactions, its further reaction to melanine must involve neither protons nor oxygen. However, a contribution of a *catalytic amount* of hydroxyl radicals from the cleavage of an extremely small amount of the hydrogen peroxide produced is a possible candidate for initiating this polymerisation reaction.

From (5) and allowing for the further (fast) consumption of an O_0 molecule, the rate of disappearance of O_2 is given by (6).

$$-d[O_2]/dt = 2k_1\ [HLH][O_2]. \qquad (6)$$

Over the pH-range 7.0-8.5 dopamine is mainly protonated, so that the total concentration of dopamine, $[L]_T$, is given by (7) and hence making use of the

$$[L]_T \approx [H_2LH^+] \qquad (7)$$

microconstant for the deprotonation of the phenolic hydroxo-group, K_m^{OH}, (6) becomes (8). The constant A in (3) above is therefore $2k_1K_m^{OH}$ and, using the

$$-d[O_2]/dt = 2k_1\ K_m^{OH}[L]_T[O_2]/[H^+] \qquad (8)$$

value for the microconstant given above, this yields a value k_1 of 0.47 ± 0.05 $dm^3\ mol^{-1}\ s^{-1}$ at 25°C.

Spectra. Typical spectra recorded during the course of the reaction are shown in Figs. 4 and 5 in absence and presence of iron(III), respectively. It can be seen that i) general

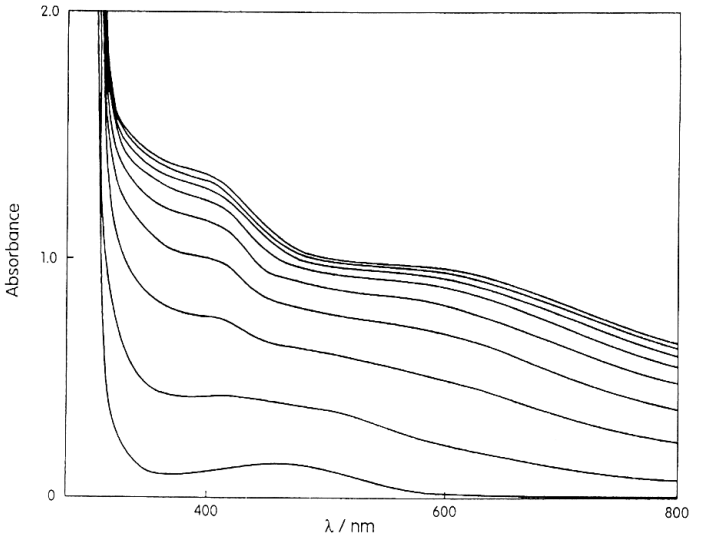


Fig. 4 - Time-dependent UV-Vis-Spectra during the autoxidation of dopamine. Spectra were recorded every 300 s, the first (bottom) one 200 s after the start of the reaction. $[DAM]_T=1.00.10^{-2}$ mol.dm^{-3}, $[O_2]_0=1.32.10^{-3}$ mol.dm^{-3}, pH 7.65, I=0.100 mol.dm^{-3}, T=298 K.

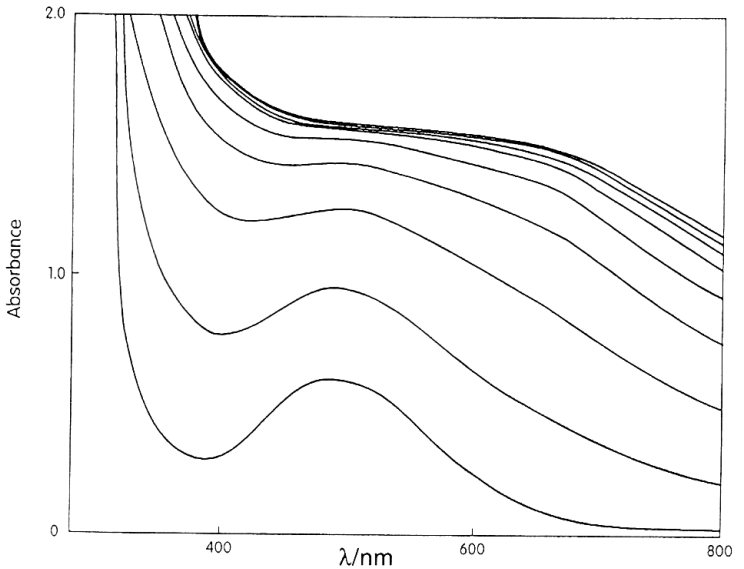


Fig. 5 - Time-dependent UV-Vis-Spectra during the autoxidation of dopamine in the presence of $1.0.10^{-4}$ mol.dm^{-3} iron(III). Experimental details are given in Fig. 4.

absorption increases due to the formation of colloidal melanine, ii) a peak at 480 nm attributable to dopaminochrome appears in the initial stages of the reaction but then disappears, iii) peaks at about 405 and 620 nm appear in the final stages of the reaction.

However, it is interesting to note that the former is suppressed in the presence of iron(III)-ions, whereas the latter is unchanged. Also it can be seen that the peak at 495 nm, due to $Fe(LH)_2^{\ddagger}$, disappears during the reaction. Since dopamine is in large excess and its complex a good indicator for iron(III), it can be concluded that iron is removed from solution, certainly by inclusion into the melanine (adsorption of iron at the surface of melanin can only account for 5% of the total iron present under our conditions[11]).

It may be of interest that it was found that UV-light (λ<300 nm) suppresses the polymerisation (Reaction 5 in Fig. 3). Furthermore, iron(III) acts as a quencher for this suppression in the wavelength range from 300 to 350 nm. Both of these effects are no doubt related to the free radical character of the melanine-forming reactions.

Interaction of iron(II) with H_2O_2 in the presence of dopamine.

To samples of dopamine, iron(II) and hydrogen peroxide were added and the resulting mixture was analysed by HPLC as described above; a successful separation and identification of DA, 5-OHDA, and 6-OHDA from the reaction mixture was achieved. A representative chromatogram is shown in Fig. 6. (Detection limits were at the pg level.)

Stopped-flow investigations of the electron transfer between iron(III) and the dopamines.

Fig. 7 shows an example of the time-dependent spectra obtained from the anaerobic interaction of iron(III) with dopamine. It shows clearly the rapid formation of the iron dopamine complex which then (rather slowly) decomposes by

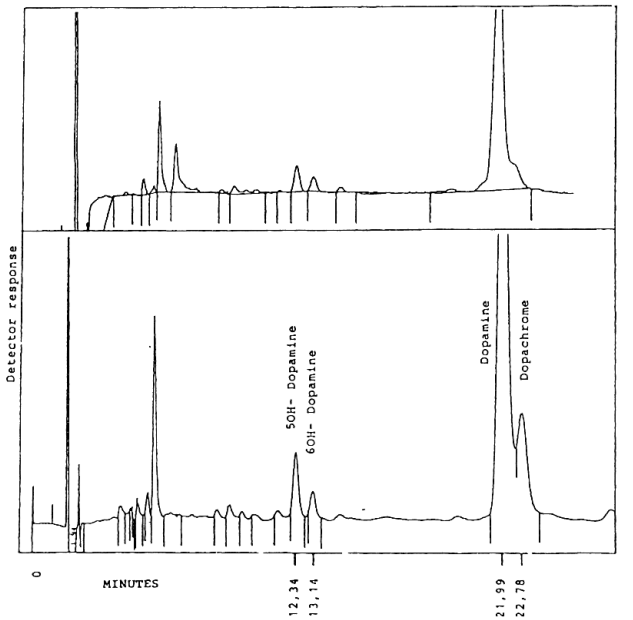


Fig. 6 - Time course of the formation of 5-OHDA and 6-OHDA as effect of iron(II) (1 mM) in a mixture of DA (1 mM) and H_2O_2 (1 mM) at 298 K after 5 (upper trace) and 20 (lower trace) minutes, respectively. pH is 7.5 in physiological NaCl-solution.

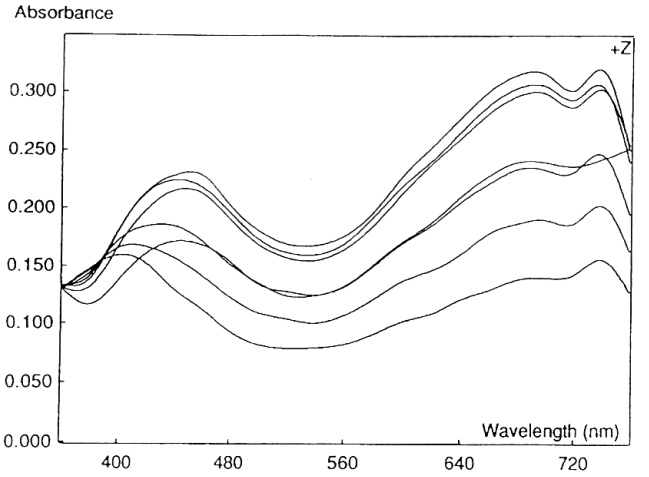


Fig. 7 - Time dependent spectra of the reaction of DA (3.0 mM) with iron(III) (0.25 mM); pH 2.49 in 0.1 M KCl. Spectra taken after 0.625, 1.25, 2.50, 5.0, 18.75, 31.25 and 50 s, respectively. Rate of electron transfer: $2.25.10^{-2}$ s^{-1}.

means of internal electron transfer yielding iron(II) and dopamine semiquinone (See Fig. 8). A similar result follows from the use 5-OHDA (Fig. 9), although the reactions are very much faster – at least partly due to statistical factors arising from the symmetry of the 5-OHDA molecule.

Fig. 10 shows the results obtained when 6-OHDA is used and it is quite clearly seen that no initial formation of an iron(III) complex is involved in the oxidation of the 6-OHDA.

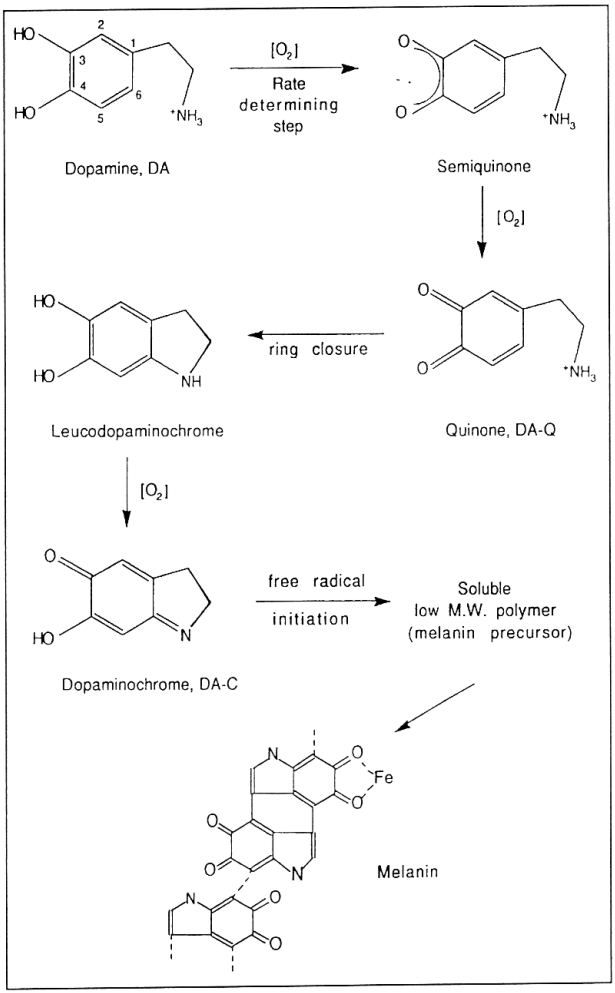


Fig. 8 - Observable products during the stepwise oxidation of dopamine (the oxidations of the related catecholamines follow a similar pattern).

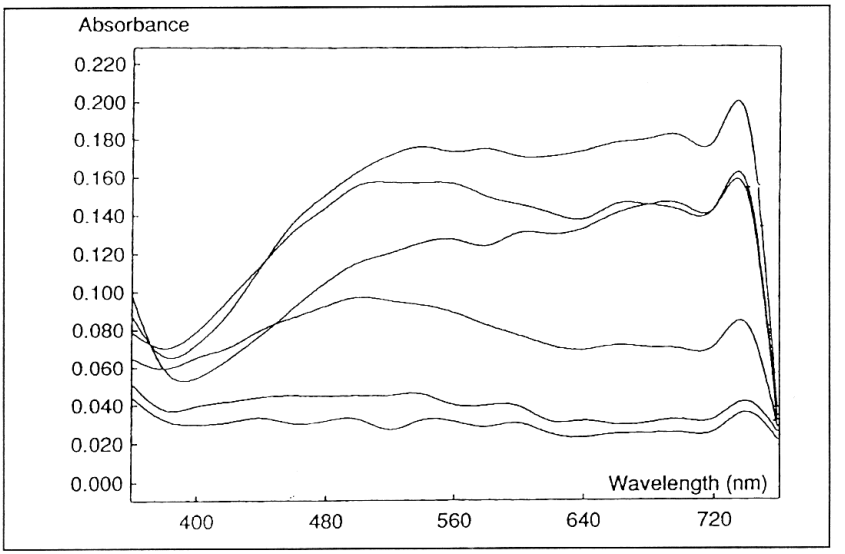


Fig. 9 - Time dependent spectra of the reaction of 5-OHDA (3.0 mM) with iron(III) (0.25 mM); pH 2.53 in 0.1 M KCI. Spectra taken after 0.625, 1.25, 2.50, 5.0, 7.50 s, respectively. Rate of electron transfer: $4.44.10^{-1}$ s^{-1}.

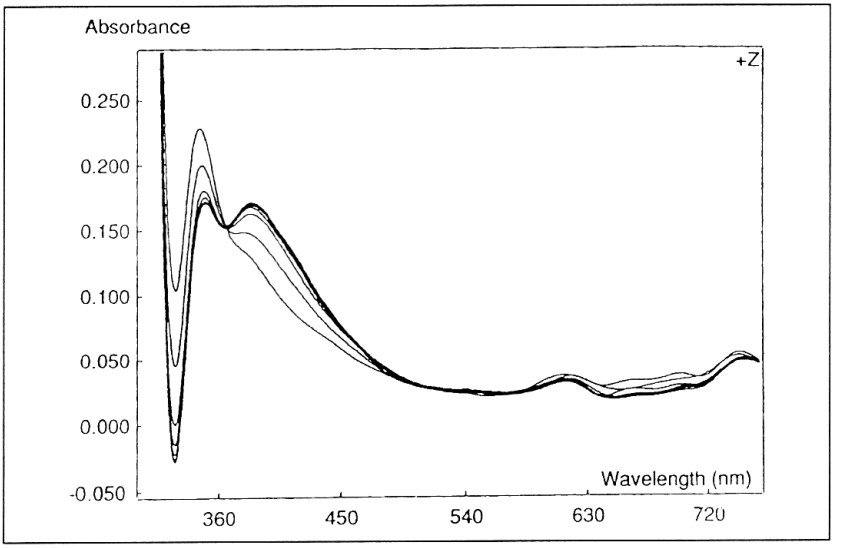


Fig. 10 - Time dependent spectra of the reaction of 6-OHDA (3.0 mM) with iron(III) (0.25 mM); pH 2.49 in 0.1 M KCI. Spectra taken after 0.25, 0.625,1.25, 2.50, 5.0 and 7.50 s, respectively. Rate of electron transfer: 600 M^{-1} s^{-1}.

Discussion.

Conclusions concerning the spontaneous autooxidation of Dopamine in the absence of metal ions.

Dopamine reacts spontaneously with dioxygen at neutral to alkaline pH via hydrogen abstraction which is rate determining. The subsequent reactions leading to dopaminochrome are comparatively fast and could not therefore be investigated. The degradation of the latter to the polymeric melanine requires neither dioxygen nor protons but might well require free-radical initiation, perhaps by a catalytic amount of OH-radical. Hydrogen peroxide is a main product of the reaction, even in the presence of iron, as proven by the recovery of a stoichiometric amount of O_2 after addition of catalase to the final solution.

Effect of metal ions on the oxidation of dopamine by dioxygen.

Metal ions such as Fe(III), Cu(II) or VO^{2+} have little if any influence on the rate of autoxidation. This might be due to the fact that, as exemplified with iron(III) an added metal ion becomes encapsulated by an inert, polymeric but soluble, precursor of melanin and eventually metal ion-containing melanin is precipitated. Neither melanin itself nor iron-containing melanin has any effect on the rate of oxidation at neutral pH as shown in Fig. 11.

Of the other metal ions examined, in view of the sensitivity of the dopaminergic neurons to manganese(II)[25,26], it is of interest to note that manganese(II) alone acts as a true catalyst[2]. The kinetics of this reaction are also interesting in that two parallel catalytic paths are followed, one being first-order and the other half-order in $[O_2]$. Furthermore, the manganese remains catalytically active throughout the reaction and thus it seems highly likely that reaction does not lead to the

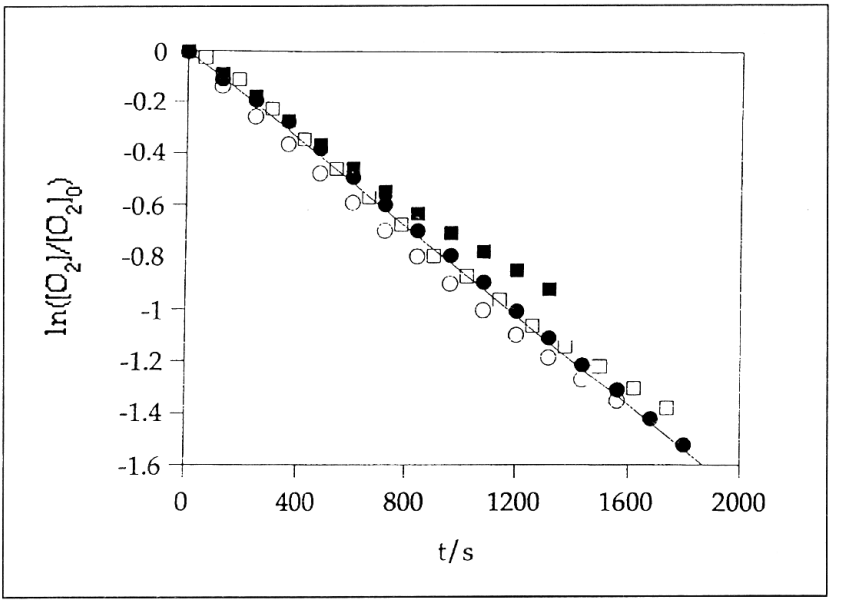


Fig. 11 - Semilogarithmic concentration/time diagram for the autoxidation of DA.10 mM DA alone (●), and synthetic melanin (o), iron(III) (❑) and iron(III)-containing melanin (■) added; pH is 7.6 in 0.1 M KCI.

formation of a potentially entrapping melanine precursor as is the case with iron(III). It is therfore highly relevant to note that, in contrast to other transition metal ions, manganese is not accumulated in neuromelanin[28].

In acid solution and in the absence of metal ions DA is unaffected by the presence of oxygen. However, iron(III) and other metal ions *initiate* a reaction, but are not a true catalyst in that reaction ceases when one mole of iron(III) is consumed per mole of dioxygen. These findings indicate that Halliwells generalisation[27] that transition metal ions *generally* catalyse autoxidation reactions of the catecholamines is not sustainable. Further work on this and the stoichiometric reaction of iron(III), dopamine and dioxygen at slightly acid pH is in progress at our institute.

A possible mechanism of the progress of Parkinson's Desease:

These *in vitro* experiments are important since they confirm: (1) that the presence of iron(II) and H_2O_2 efficiently converts DA into the cytotoxic 6-OHDA *via* a Fenton-type reaction. (2) The stopped-flow experiments show that the oxidation of 6-OHDA by iron(III) – unlike the other dopamines – proceeds without the prior formation of a metal-ligand complex. In other words, the reaction of iron(III) with 6-OHDA is an '*outer sphere*' electron transfer reaction, whereas with the other catecholamines it is an '*inner sphere*' reaction. The reason for this difference is obviously linked to the considerable stability of the para-semiquinones and paraquinones over the respective ortho-species. This implies that 6-OHDA alone should be capable of removing iron(III) (in the reduced form of iron(II)) from complexing species such as the storage protein ferritin, and thus it is not surprising that this has actually been experimentally achieved[29]. Of equal importance is the observation that, *via* the Fenton related reaction, the 6-OHDA involved is readily regenerated by hydroxylation of DA – i.e. leading to a continuous production of cytotoxic species. These hydroxylation reactions are obviously related to the occurrence of 5-OHDA and 6-OHDA found in human urine – especially that of patients undergoing dopa therapy[30].

The trapping of iron(III) by melanin as demonstrated by the reaction of DA with O_2 in the presence of iron(III) and its subsequent complete removal from the system strongly suggests that one the functions of melanin formation in the SN is the entrapment of any free iron(III) to provent its toxic effects. These ideas are summarized in Fig. 12.

Finally, the true catalytic effect of manganese(II) is obviously directly related to its toxic effects, since it can effect the oxidation of DA without subsequently being effectively removed from the system as is the case with iron.

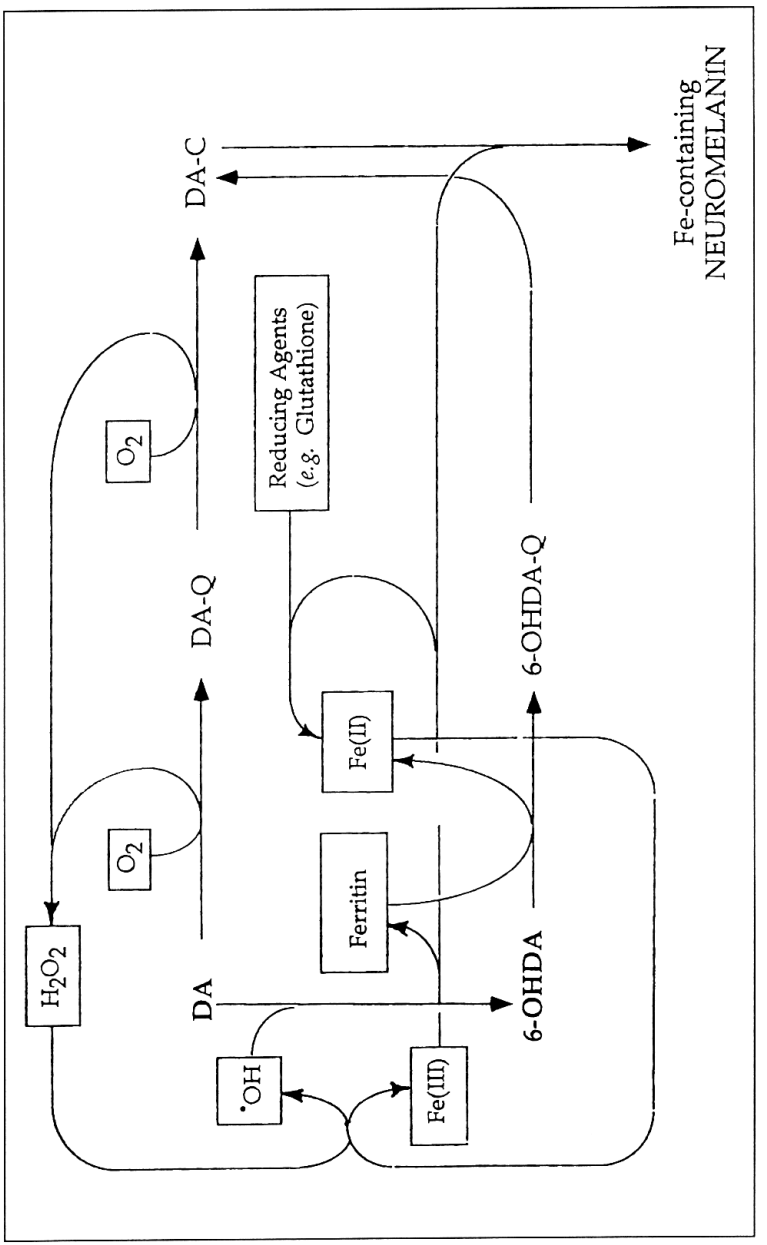


Fig. 12 - Implications of the ability of 6-OHDA to liberate iron from ferritin.

We are therefore continuing our studies of these *in vitro* chemical reactions considered to be important for the development of PD believing this wil provide a sound chemical basis for the further *in vivo* studies that are required in order to clarify the mechanisms involved in the biology of ageing.

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